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Efficiency of Beef Extract for the Recovery of Poliovirus from Wastewater Effluents

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Efficiency of Beef Extract for the Recovery of Poliovirus from Wastewater Effluents

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The efficiency of poliovirus elution from fiber glass cartridge filters (K27), epoxy-fiber glass-asbestos filters (M780), and pleated cartridge filters was assessed by using 3% beef extract (pH 9.0) or 0.1 M glycine (pH 11.5). Poliovirus type I, strain LSc, was seeded into 20- to 25-gallon (ca. 75.6- to 95.6-liter) samples of treated sewage effluent and concentrated by using a filter adsorption-elution technique. Virus elution was accomplished by using either two 600-ml portions of 3% beef extract (pH 9.0), or two 1-liter portions of 0.1 M glycine (pH 11.5). In all experiments, beef extract elution followed by organic flocculation was found to be superior, yielding a mean recovery efficiency of 85%, with recoveries ranging from 68 to 100%. Elution with 0.1 M glycine (pH 11.5) followed by inorganic flocculation resulted in a mean recovery efficiency of 36%. The variable range of recoveries with beef extract could not be significantly improved by varying the type of beef extract or by extending the elution time to 30 min. Second-step reconcentration of 1-liter seeded sewage effluent and renovated wastewater samples indicated that organic flocculation was a more efficient method for virus recovery than inorganic flocculation. Beef extract concentrations of less than 3% were found to be efficient in the recovery of poliovirus from renovated wastewater.

A number of techniques have been used for the recovery of human enteroviruses from various aquatic systems, including gauze-pad adsorption (3), polyelectrolyte adsorption (13), polyethylene glycol hydroextraction (18), and filter adsorption-elution (23). The latter technique has been adopted and modified by a number of investigators for the recovery of enterovirus from drinking water (8), estuarine water (16), treated sewage (20), and seawater (7). Although the filter adsorption-elution method is one of the most reliable, sensitive, and widely used techniques (10), recovery efficiencies, as judged by seeding experiments using known concentrations of virus, exhibit great variability. Sobsey et al. (19), studying the detection of seeded poliovirus in tap water, reported an average recovery efficiency of 77%. In a series of five experiments utilizing fiber glass cartridge and membrane filters as virus adsorbents, they reported recoveries ranging from 48 to 95%. Jakubowski et al. (12), using an epoxy-fiber glass Balston filter, observed recovery efficiencies ranging from 42 to 52% for poliovirus-seeded potable water. In both studies mentioned above, 0.05 M glycine (pH 11.5) was used to elute filter-adsorbed viruses.

Although glycine has been recommended as the standard eluant for the recovery of enteric viruses from water and wastewater (1), a number of alkaline proteinaceous substances have been successfully used for this purpose, including bovine serum-supplemented media (24), beef extract (2, 25, 26), and nutrient broth (9). Recently, Katzenelson et al. (14) reported the elution of virus from an epoxy-fiber glass-asbestos filter with 3% beef extract (pH 9.0). In a series of comparative experiments using tap water seeded with poliovirus type 1, beef extract elution was found to be superior to glycine elution, yielding an average efficiency of 74.4%, with recoveries ranging from 60 to 91%. Elution with 0.05 M glycine (pH 11.5) showed a 35% mean recovery efficiency with a range of 30 to 47%.

Initial experiments in our laboratory, using a filter adsorption-elution system with a 0.10 M glycine (pH 11.5) eluant resulted in erratic virus recoveries in seeded wastewater experiments. In an effort to increase the recovery efficiency of the system, a study was conducted to determine the efficiency of the beef extract elution method. In this report, we apply the findings of Katzenelson et al. (14) to the elution of poliovirus from seeded wastewater samples.

MATERIALS AND METHODS

Virus. Plaque-purified poliovirus type 1 (LSc) was propagated on Buffalo green monkey kidney cells (BGM) (4), and prepared by the procedure of Jaku-

bowski et al. (11). This technique was used to remove virus aggregates and obtain a monodispersed culture.

Virus assay. Samples were treated with chloroform for 30 min and diluted in phosphate-buffered saline (pH 7.2). A 0.5-ml amount of sample was inoculated onto BGM monolayers in 25-cm^2 flasks. Viruses were allowed to adsorb for 60 min at 30°C with rocking. The inoculum was then decanted, and 4 ml of a neutral red agar overlay (10) was added to each flask. Flasks were then incubated at 36°C and observed for plaques over a 7-day period.

Virus concentrations. A known concentration of poliovirus was added to 20- to 25-gallon (ca. 75.6- to 95.6 liter) volumes of tertiary-treated (denitrification-filtration) sewage obtained from the 12-Pines Sewage Treatment Plant, Medford, N.Y. Initial virus concentration was carried out by means of an Aquella virus concentrator (Carborundum Co., Niagara Falls, N.Y.), following the procedures outlined by Farrah et al. (5), with the exception that no clarifying filters were used.

Glycine elution. Glycine buffer (0.10 M) was prepared and adjusted to pH 11.5 by using 10 N NaOH. The pH meter had previously been standardized by using a saturated calcium hydroxide solution (pH 12.5) to insure an accurate glycine pH of 11.5 (5). Polioviruses adsorbed to concentrating filters were eluted by using two 1-liter volumes of glycine buffer (22), a process which required 3 to 5 min. The eluates were collected in equal volumes of pH 2.0 glycine buffer and neutralized immediately (pH 7.0). A second-stage virus reconcentration was accomplished by an aluminum hydroxide flocculation procedure (5).

Beef extract elution. Viruses were eluted from the virus-concentrating filters by using two 600-ml volumes of 3% beef extract (pH 9.0). Reconcentration techniques were carried out by the procedure of Katzenelson et al. (14). The pH was lowered to 3.5, causing the formation of a virus-adsorbing protein precipitate. The precipitate was then collected by centrifugation at 5,000 × *g* for 10 min. The resulting pellet was dissolved in a small volume (20 to 35 ml) of 0.15 M Na_2HPO_4 (pH 9.0) and adjusted, if necessary, to a final pH of 7.2 by using dilute acid or base. Samples were stored at −70°C until assayed.

RESULTS

Efficiency of beef extract in the recovery of virus. A comparison of the efficiency of virus recovery, using 3% beef extract (pH 9.0) or 0.10 M glycine (pH 11.5) was assessed in poliovirus-seeded 25-gallon volumes of treated sewage effluent. The viruses were concentrated via filter adsorption-elution with a fiber glass cartridge depth filter (K27) and epoxy-fiber glass-asbestos filters (porosity, 1.0 and 0.45 μm). Clarifying filters were not used in any of the experiments because the turbidity of the tertiary-treated sewage was quite low. The physical and chemical characteristics of the sewage are shown in Table 1.

The results of duplicate experiments (Table 2) indicated that beef extract elution followed by organic flocculation was superior to a glycine elution-inorganic flocculation method in the recovery of seeded poliovirus from sewage effluent. Beef extract elution yielded a recovery efficiency of >100 and 96%, respectively, in two trials, whereas the recovery efficiency with glycine was 31 and 41%, respectively.

TABLE 1. *Characteristics of 12-Pines tertiary-treated sewage used in seeding experiments*

Characteristic[a]	Mean	Range
Turbidity (NTU)	4.58	1.2–9.0
Conductivity (μmho)	439.50	393–500
pH	6.61	6.10–7.20
Total alkalinity	95.38	48–115
Chloride	54.13	47–64
Sulfate	35.63	28–54
Nitrate-nitrogen	3.38	0.07–16.0
Nitrite-nitrogen	0.11	0.006–0.49
Ammonia-nitrogen	5.34	0.06–15
Total Kjeldahl nitrogen	6.63	0.9–17
Orthophosphate	6.13	4.8–6.8
Fe^{3+}	<0.05	<0.05
Mn^{2+}	0.02	0.01–0.03
Mg^{2+}	4.53	2.7–7.8
Ca^{2+}	19.0	15–24
Na^{+}	58.25	54–68
K^{+}	12.25	11–13
Total suspended solids	5.00	1–14
Total organic carbon	15.13	10–29

[a] Characteristics, unless otherwise indicated, are in milligrams per liter. NTU, Nephelometric turbidity unit.

To insure reproducibility, a series of additional seeding experiments was carried out, using the techniques previously described. The data (Table 3) show a consistently high recovery of poliovirus from wastewater when beef extract was used. In seven trials, the mean recovery efficiency was 85%, with recoveries ranging from 68 to 100%.

Additional studies were conducted to determine whether an extension of the contact time between the virus-concentrating filters and the eluant would increase the efficiency of virus recovery. Comparative experiments were performed by seeding two 25-gallon wastewater samples with identical concentrations of poliovirus (1.54×10^7 plaque-forming units [PFU]). One sample was eluted in the manner previously described, whereas the other sample was eluted after allowing the beef extract to remain in contact with the virus-laden filters for a total of 30 min. Both samples were reconcentrated by flocculation. Immediate elution resulted in the recovery of 1.34×10^7 PFU (87%), whereas the extended elution method yielded a recovery of 1.24×10^7 total PFU (80.5%). These results suggested that the elution was likely instantaneous and also that poliovirus was not inactivated by prolonged contact (30 min) with beef extract at pH 9.0.

TABLE 2. *Comparison of poliovirus recovery: 3% beef extract (pH 9.0) versus 0.10 M glycine (pH 11.5)*

Expt no.	Total PFU recovered from:[a]			
	Beef extract elution		Glycine elution	
	Input	Reconcentrate	Input	Reconcentrate
1	3.03×10^5	3.16×10^5 (>100)	5.68×10^5	1.80×10^5 (31.69)
2	3.31×10^6	3.18×10^6 (96.07)	2.27×10^6	9.30×10^5 (40.96)

[a] Values in parentheses indicate percent recovery based on input values.

TABLE 3. *Overall efficiency of poliovirus recovery by using 3% beef extract (pH 9.0) elution-reconcentration*

Expt no.	Total PFU recovered from:		% Recovery
	Input	Reconcentrate	
1	3.03×10^5	3.16×10^5	100.0
2	3.31×10^6	3.18×10^6	96.07
3	1.45×10^7	1.22×10^7	84.13
4	2.33×10^7	1.82×10^7	78.11
5	1.54×10^7	1.05×10^7	68.18
6	1.54×10^7	1.21×10^7	78.57
7	5.30×10^6	4.95×10^6	93.39
Mean			85.49
Range			68–100

TABLE 4. *Efficiency of poliovirus recovery from pleated cartridge filters using 3% beef extract (pH 9.0)*

Expt no.	Total PFU recovered from:	
	Input	Reconcentrate[a]
1	3.28×10^6	2.04×10^6 (62.19)
2	2.22×10^6	1.53×10^6 (68.91)
Mean		(65.55)

[a] Values in parentheses indicate percent recovery based on input.

TABLE 5. *Comparison of the efficiency of reconcentrating seeded poliovirus by inorganic or organic flocculation methods*

Expt no.	Total PFU recovered after:[a]	
	Inorganic flocculation	Organic flocculation
1	2.35×10^5 (68.11)	3.04×10^5 (88.11)
2	2.07×10^5 (60.00)	2.87×10^5 (83.18)
Avg	(64.05)	(85.64)

[a] Values in parentheses indicate percent recovery based on an input value of 3.45×10^5 total PFU.

Recently, a number of investigators have adopted pleated cartridge filters for use in the recovery of enteroviruses from large-volume water samples (5, 7, 16). The increased surface area of these filters eliminates clogging problems, allowing higher volume samples to be processed. Experiments were therefore performed to insure that 3% beef extract (pH 9.0) was equally effective in systems using these filters. Seeded sewage effluent samples were processed through a filter series consisting of a fiber glass K27 filter and a 0.45-μm pleated filter (Duo-Fine; Filterite, Timonium, Md.). The results (Table 4) indicated that an average of 65% of the input polioviruses were recovered after concentration and reconcentration.

Efficiency of beef extract in second-stage reconcentration. Katzenelson et al. (14) recently reported that an organic flocculation procedure utilizing 3% beef extract was superior to an inorganic flocculation method that used glycine for the recovery of seeded poliovirus from tap water during second-stage reconcentration. To determine whether a similar occurrence could be observed in seeded wastewater, poliovirus was inoculated into 1-liter volumes of treated sewage effluent. The sample was divided into 250-ml portions, and the poliovirus was concentrated by either organic or inorganic flocculation. The results (Table 5) support the findings of Katzenelson et al. (14) regarding the superiority of beef extract in the second-step reconcentration process. Of the input viruses, 85% were recovered with the organic method, whereas inorganic flocculation yielded an average recovery of 64%.

Since all previous reconcentration experiments utilized 3% beef extract, it was of interest to determine whether lower concentrations of beef extract would yield similar virus recoveries. Initial experiments were conducted with small volumes of renovated wastewater collected from observation wells located beneath wastewater recharge basins. Six-liter volumes were seeded with poliovirus and dispensed into 1-liter portions. Samples were concentrated by organic flocculation, using beef extract at final concentrations of 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0%, respectively. The results of this experiment (Table 6) indicated that lower concentrations of beef extract also yielded satisfactory virus recovery.

Since beef extract concentrations of less than 3% appeared to be effective in a reconcentration process, it was of interest to test their relative efficiencies of virus elution from concentrating filters. In a series of preliminary experiments,

TABLE 6. *Reconcentration efficiency of various concentrations of beef extract*

% Beef extract	Total PFU recovered from:[a]		Mean recovery (%)
	Expt 1	Expt 2	
0.5	1.14×10^5 (74.03)	1.99×10^5 (80.56)	77.29
1.0	1.10×10^5 (71.42)	2.28×10^5 (92.30)	81.86
1.5	8.84×10^4 (57.40)	2.47×10^5 (100)	78.70
2.0	1.60×10^5 (100)	2.48×10^5 (100)	100.0
2.5	1.36×10^5 (88.31)	1.67×10^5 (67.61)	77.96
3.0	1.12×10^5 (72.72)	2.67×10^5 (100)	86.36

[a] Values in parentheses indicate percent recovery based on input values of 1.54×10^5 for the first experiment and 2.47×10^5 for experiment 2.

poliovirus was seeded into 25-gallon wastewater effluent samples and concentrated by using a fiberglass cartridge-pleated filter series. Elution was accomplished by using either 1% or 3% beef extract (pH 9.0). Preliminary results indicated that 1% beef extract was as efficient as 3% beef extract in the recovery of poliovirus. Elution with 3% beef extract resulted in the recovery of 68% of the input virus, whereas 75% of the viruses were recovered by using 1% beef extract elution. These results were obtained with the low-turbidity sewage previously described. It is not presently known whether similar findings would be obtained, should lower quality effluents be used.

DISCUSSION

A number of workers have shown 3% beef extract to be effective in the elution of enteric virus from a variety of concentrating filters (2, 13, 17). Recently, beef extract was found to be a more efficient eluant than 0.05 M glycine (pH 11.5) for the recovery of poliovirus type 1 in second-stage virus reconcentration procedures (14). The results presented in this paper appear to support these findings and to extend their use to fiber glass cartridge depth filters (K27), epoxy-fiber glass-asbestos microfilters (Cox-M780), and pleated cartridge filters. In a series of comparative experiments, poliovirus type 1 seeded into tertiary-treated sewage was recovered from virus-adsorbing filters with an average efficiency of 85%, using a 3% beef extract eluant. Elution with pH 11.5 glycine resulted in a mean recovery efficiency of 36%. These values are consistent with those reported by Katzenelson et al. (14) for recovery of virus from seeded tap water.

It is not known whether the decreased efficiency of glycine elution in our system was the result of viral inactivation caused by the high pH of the glycine (pH 11.5). This pH appears to be necessary for efficient virus elution (21) and has not been reported to be viricidal for poliovirus during the time required for filter elution and subsequent neutralization (15). However, Jakubowski et al. (11) have suggested that a lowering of the pH may reduce the risk of viral inactivation, particularly since pH inactivation data on other members of the enterovirus group are unavailable. Glycine solutions having pH's of 9.8 and 10.5 have been shown to be effective in the elution of seeded poliovirus from Balston and nitrocellulose membrane filters (11), and from pleated filters when using tap water (7). Fields and Metcalf (6) reported that glycine having a pH of 10.5 to 11.5 could not be used for the elution of adenovirus type 5 from adsorbing filters because of the pH sensitivity of the virus. They found that 3% beef extract at pH 9.0 yielded a recovery efficiency of 90% during adenovirus seeding experiments in artificial or natural seawater.

Although the recovery efficiencies of seeded poliovirus were quite high with beef extract, they varied from 68 to 100% in individual experiments. Efficiencies were not significantly improved by extending the time of elution or by utilizing a different type of beef extract. Variations in recovery efficiencies could have been due to differences in sample water quality during each experiment (11).

In a limited number of trials, the average efficiency of poliovirus recovery with a fiber glass cartridge-pleated filter series was somewhat lower than those recoveries observed with a fiber glass cartridge-membrane filter series. It is not known whether the higher efficiencies noted in the latter series were due to improved viral adsorption during concentration or to the greater elution capabilities of these particular filters. In spite of slightly lower recovery values, the pleated filter series would appear to be most practical for the processing of large-volume water samples because of their ability to resist clogging.

Preliminary experiments using small-volume seeded well water samples indicated that concentrations of beef extract lower than 3% might prove to be effective in the recovery of viruses from second-step reconcentration processes. Of the input viruses, 81% were recovered by using 1% beef extract, whereas 100% of the viruses were reconcentrated and recovered with 2% beef extract. Preliminary experiments also indicated that 1% beef extract was as efficient as 3% in the elution of poliovirus from virus-absorbing filters. This laboratory is presently conducting extensive field analyses for the presence of enterovirus in wastewater effluents and renovated well water by the beef extract elution methods described above.

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